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# Supporting Information

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for

## Synthesis of a Universal 5-Nitroindole Ribonucleotide and Incorporation into RNA by a Viral RNA-Dependent RNA Polymerase

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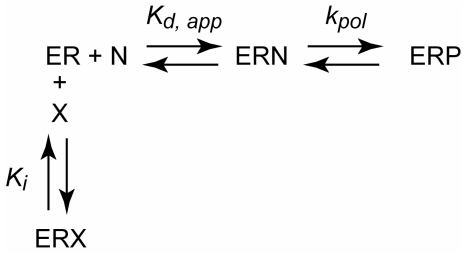



**Figure S1.** Model of competitive inhibition used for the kinetic simulation. ER: $3D^{pol}$-s/s template complex; N: NTP; X: NTP analogues such as **5** and RTP.

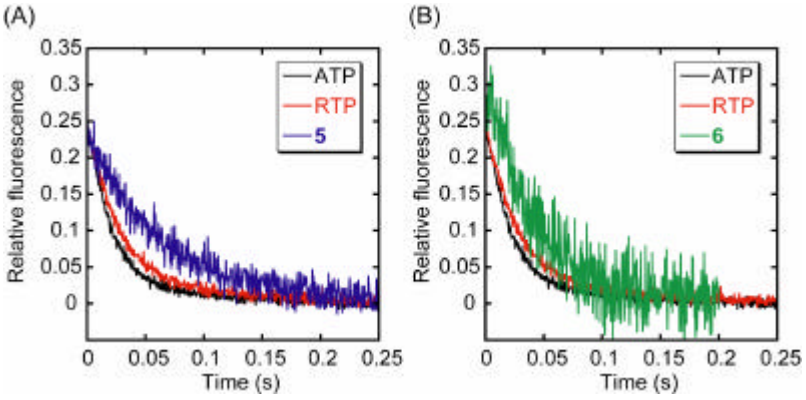
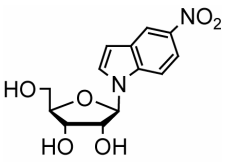



**Figure S2**. Raw fluorescence data obtained by stopped-flow kinetic studies of inhibition of incorporation of ATP catalyzed by PV RdRP. [$3D^{pol}$] = 0.5 µM, [s/s-U-2AP] = 0.25 µM (duplex). [ATP], [RTP], [**5**] and [**6**] = 100 µM. A) Combined fluorescence data for ATP, RTP, and **5**. B) Combined fluorescence data for ATP, RTP, and **6**.

## Chemical Synthesis

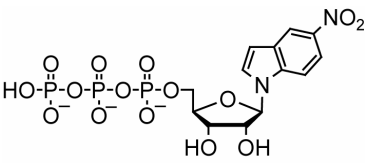
**General.** Chemical reagents were obtained from Acros or Aldrich. Solvents were from Fisher Scientific. Triethylammonium acetate (TEAA, pH 7 at 1 M) and triethylammonium bicarbonate (TEAB, pH 8.5 at 1 M) buffers were from Fluka. Unless otherwise noted, reactions were performed under an atmosphere of dry argon or nitrogen. Commercial grade reagents were used without further purification unless specifically noted. Dichloromethane and acetonitrile were distilled from calcium hydride under nitrogen. Buffers for HPLC purification and analysis were prepared and diluted with distilled and deionized water. Column chromatography employed ICN SiliTech silica gel (32-63 µm). Preparative HPLC purification employed an Agilent 1100 series

instrument (preparative scale) with the following column/eluant combinations: (*Prep HPLC Purification Method I*) column: Aquasil C18 preparative column (21.2 x 250 mm, 5 µm; Thermo Electron Corporation), mobile phase method: (flow rate = 20 mL/min) linear gradient of 10% to 20% $CH_3CN$ in TEAA buffer (0 to 20 min, 20 mM TEAA, pH 6) followed by 20% to 90% $CH_3CN$ in TEAA buffer (20 to 30 min, 20 mM TEAA, pH 6); (*Prep HPLC Purification Method II*) column: Atlantis C18 preparative column (19 x 150 mm, 5 µm; Waters Corporation), mobile phase method: (flow rate = 25 mL/min) linear gradient of 10% to 30% $CH_3CN$ in TEAA buffer (0 to 20 min, 20 mM TEAA, pH 6) followed by 20% to 90% $CH_3CN$ in TEAA buffer (20 to 30 min, 20 mM TEAA, pH 6). The purity of each 5'-phosphate was determined by analytical HPLC analysis (254 nm wavelength observed) of the concentrated, purified material. Analytical HPLC analysis was performed on a Hewlett-Packard 1100 series instrument (analytical scale) equipped with an Aquasil C18 column (4.6 x 250 mm, 5 µm) running the following mobile phase: (flow rate = 1 mL/min) linear gradient of 1 to 80 % $CH_3CN$ in $KH_2PO_4$ buffer (0 to 40 min, 100 mM $KH_2PO_4$, pH 6). Nuclear magnetic resonance (NMR) spectroscopy employed Bruker AMX-360 and DRX-300 MHz spectrometers. Internal solvent peaks were referenced in each case. Chemical shifts for $^{13}C$ NMR and $^{31}P$ NMR analyses performed in $D_2O$ were indirectly referenced to 10% acetone in $D_2O$ ($CH_3$ set to 30.89 ppm)[1] and 85% $H_3PO_4$ (0 ppm), respectively. $^{13}C$ chemical shifts for nucleoside triphosphates denoted with an asterisk fail to resolve into clean singlets due to apparent conformational restriction. Mass spectral data were obtained by The University of Texas at Austin and The Pennsylvania State University Mass Spectrometry Facilities. Elemental analyses were performed by Midwest Microlab, LLC (Indianapolis, IN). Melting points are uncorrected.




**1-β-D-ribofuranosyl-5-nitroindole (3).** To a degassed solution of β-D-ribofuranose-1-acetate-2,3,5-tribenzoate (**9**, 5.91 g, 11.71 mmol) in $CH_2Cl_2$ (70 mL) was added a solution of $TiCl_4$ in $CH_2Cl_2$ (13 mL, 1.0 M).[2] The reaction was stirred at 23 °C for 2 h, followed by quenching with deionized $H_2O$ (75 mL). The organic layer was separated, washed with deionized $H_2O$ (1 x 75 mL), dried over anhydrous $MgSO_4$, and concentrated in vacuo. The crude material was dissolved in $CH_3CN$ (20 mL) and used without further purification in the subsequent coupling step. Separately, a suspension of 5-nitroindole (6.17 g, 38.05 mmol) in $CH_3CN$ (20 mL) was added to a suspension of NaH

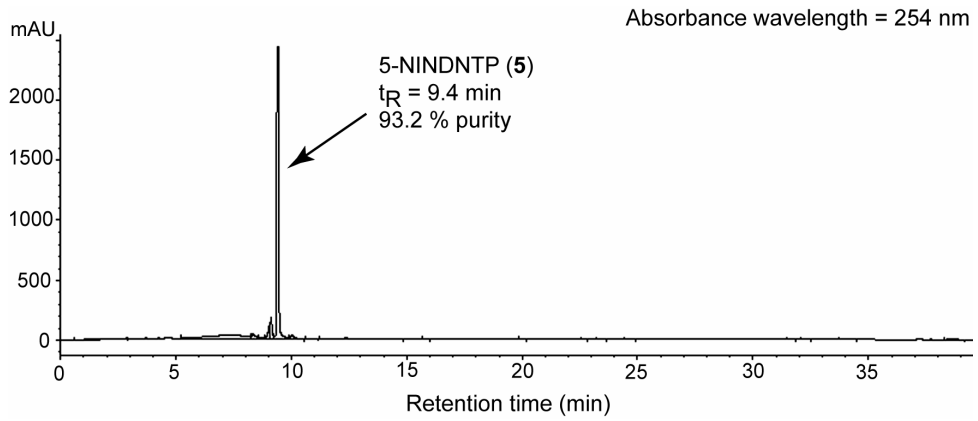
(95% dry, 556.3 mg, 23.18 mmol) in $CH_3CN$ (80 mL) at 2 °C. To this solution was added crude chlorosugar **10** via cannula followed by additional $CH_3CN$ (10 mL). The reaction was warmed to 23 °C and stirred for 22 h. The crude product was then poured into saturated aqueous $NH_4Cl$ (250 mL) and EtOAc (250 mL) was added. The organic layer was separated, washed with saturated aqueous NaCl (250 mL), dried over anhydrous $MgSO_4$, and concentrated in vacuo. The crude material was partially purified by column chromatography (20 % EtOAc in hexanes) and used directly in the next step. A pressure tube was charged with the reaction products and $NH_3$ in MeOH (80 mL, ~7 N). The tube was sealed and heated to 50 ºC for 12 h. The tube was cooled, vented, and the contents concentrated in vacuo. The crude material was purified first by column chromatography (step gradient: 5 % MeOH in $CH_2Cl_2$ to 10 % MeOH in $CH_2Cl_2$) and subsequently by recrystallization (compound was dissolved in 75 mL warm acetone and 75 mL $Et_2O$ was added). The light yellow precipitate was collected, washed with excess $Et_2O$, and dried in vacuo to yield ribonucleoside **3** (630.5 mg, 18 % yield over 3 steps). $^1$H NMR (360.1 MHz, [$D_6$]DMSO): *d* 8.58 (d, *J* = 2.2 Hz, 1H), 8.04 (dd, *J* = 2.3 Hz, *J* = 9.1 Hz, 1H), 7.89 (d, *J* = 3.4 Hz, 1H), 7.81 (d, *J* = 9.2 Hz, 1H), 6.83 (d, *J* = 3.4 Hz, 1H), 5.98 (d, *J* = 6.2 Hz, 1H), 5.43 (d, *J* = 6.7 Hz, 1H), 5.22 (d, *J* = 4.8 Hz, 1H), 5.08 (t, *J* = 5.2 Hz, 1H), 4.28 (m, 1H), 4.10 (ddd [app dt], *J* = 3.2 Hz, *J* = 5.0 Hz, *J* = 5.0 Hz, 1H), 3.97 (m, 1H), 3.67-3.58 (m, 2H). $^{13}$C NMR (90.6 MHz, DMSO-$d_6$): *d* 141.1, 138.7, 129.3, 127.9, 117.4, 116.7, 110.9, 104.6, 89.0, 85.4, 74.5, 70.3, 61.3. UV $\lambda_{max}$ (MeOH) 267 nm ($\varepsilon$ = 18 700 $M^{-1}$ $cm^{-1}$). IR (KBr): $\nu_{max}$ ($cm^{-1}$) 3426, 3364, 1510, 1347, 1324, 1086. mp 197–199 °C. HRMS ($CI^+$) calcd. for $C_{13}H_{15}N_2O_6$ $[M+H]^+$ 295.0930, found 295.0936. Anal. calcd. for $C_{13}H_{14}N_2O_6$: C 53.06; H 4.80; N 9.52. Found: C 53.13; H 4.78; N 9.29.

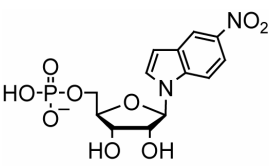



**1-β-D-ribofuranosyl-5-nitroindole triphosphate triethylammonium salt (5).** Ribonucleotide **5** was prepared by the widely-utilized "one-pot, three-step" methodology for nucleotide synthesis.[3-15] Accordingly, a solution of **3** (62.5 mg, 0.21 mmol), Proton-Sponge (1,8-bis(dimethylamino)naphthalene, 85.6 mg, 0.40 mmol), and trimethylphosphate (2.2 mL) was cooled to 2 °C. $POCl_3$ (40 μL, 0.43 mmol) was added dropwise, and the solution was stirred for 2 h. Anhydrous $Bu_3N$ (240 μL, 1.01 mmol) was injected followed by a solution of tributylammonium pyrophosphate (538.5 mg) in DMF (2 mL). The reaction was stirred for 2 min then

hydrolyzed by addition of triethylammonium bicarbonate (TEAB, 1.0 M, 5 mL). The crude reaction products were purified by preparative HPLC (HPLC purification method I) to yield triphosphate **5** as the triethylammonium salt ($t_R$ = 11-13.5 min.). This material was concentrated *in vacuo*, redissolved in TEAB (100 mM, 10 mL) and lyophilized to dryness. The material was redissolved in deionized $H_2O$ (3 mL) and lyophilized again to dryness. Triphosphate **5** was isolated as a yellow solid (49.1 mg, 24 % yield). $^1$H NMR (360.1 MHz, $D_2O$): *d* 8.37 (d, *J* = 2.2 Hz, 1H), 7.90 (dd, *J* = 2.2 Hz, *J* = 9.2 Hz, 1H), 7.63 (d, *J* = 3.4 Hz, 1H), 7.49 (d, *J* = 9.2 Hz, 1H), 6.69 (d, *J* = 3.3 Hz, 1H), 5.96 (d, *J* = 7.0 Hz, 1H), 4.54 (m, 1H), 4.38 (m, 1H), 4.18 (m, 1H), 4.09-3.99 (m, 2H), 2.97 (q, *J* = 7.3 Hz, ca. 27H, TEAA salt), 1.73 (s, ca. 3.5H, TEAA salt), 1.06 (t, *J* = 7.3 Hz, ca. 40.5H, TEAA salt). $^{13}$C NMR (90.6 MHz, $D_2O$): *d* 182.0 (TEAA salt), 142.3, 140.5, 129.4, 129.0, 119.1, 118.5, 110.9, 106.8, 88.8, 84.5, 84.4, 74.4, 71.4, 66.4*, 47.4 (TEAA salt), 24.0 (TEAA salt), 9.0 (TEAA salt). $^{31}$P NMR (145.8 MHz, $D_2O$): *d* -9.07 (br s), -10.69 (m), -21.92 (br s). HRMS (FAB$^-$) calcd for $C_{13}H_{16}N_2O_{15}P_3^-$ [*M*–TEAA+3H]$^-$ 532.9764, found 532.9775.

Analysis of **5** revealed > 93% purity:

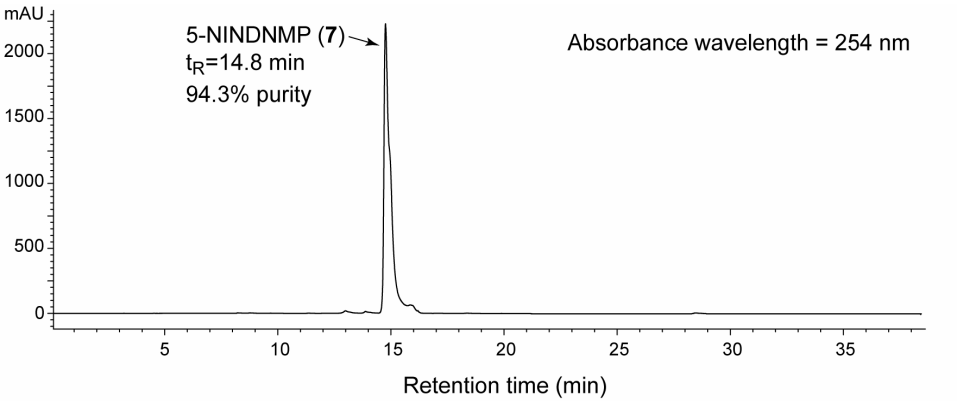


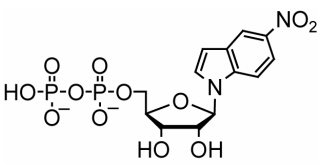




**1-β-D-ribofuranosyl-5-nitroindole monophosphate triethylammonium salt (7).** To a partially dissolved solution of **3** (66 mg, 0.22 mmoles) in trimethylphosphate (2.2 mL) was added Proton-Sponge (1,8-bis(dimethylamino)naphthalene, 83.0 mg, 0.39 mmol). The solution was cooled to 2 °C and $POCl_3$ (50 µL, 0.55 mmoles) was added dropwise. The yellow solution was stirred for 2 h at 2 °C, then quenched with triethylammonium bicarbonate (TEAB, 1.0 M solution, 5 mL).[16] This solution was lyo-

philized to dryness and purified by preparative HPLC (HPLC purification method I) to yield monophosphate **7** ($t_R$ = 14 min). The purified material was concentrated in vacuo, redissolved in deionized $H_2O$ and lyophilized to dryness to afford monophosphate **7** as a yellow, oily solid (29 mg, 24% yield). $^1$H NMR (300.1 MHz, $D_2O$): *d* 8.48 (d, *J* = 2.2 Hz, 1H), 8.00 (dd, *J* = 2.2 Hz, *J* = 9.2 Hz, 1H), 7.65 (d, *J* = 3.5 Hz, 1H), 7.58 (d, *J* = 9.2 Hz, 1H), 6.75 (d, *J* = 3.4 Hz, 1H), 6.02 (d, *J* = 7.0 Hz, 1H), 4.54 (m, 1H), 4.31 (m, 1H), 4.20 (m, 1H), 3.95 (m, 2H), 3.04 (q, *J* = 7.3 Hz, 20H, TEAA salt), 1.80 (s, 8H, TEAA salt), 1.12 (t, *J* = 7.3 Hz, 30H, TEAA salt). $^{13}$C NMR (75.5 MHz, $D_2O$): *d* 180.6 (TEAA salt), 142.0, 140.0, 128.8, 128.6, 118.7, 118.1, 110.5, 106.3, 88.5, 84.2, 84.1, 74.0, 71.1, 65.1, 47.0 (TEAA salt), 22.9 (TEAA salt), 8.6 (TEAA salt). $^{31}$P NMR (145.8 MHz, $D_2O$): *d* 1.12 (s). HRMS (ESI$^-$) calcd for $C_{13}H_{14}N_2O_9P^-$ [*M*-TEAA+H]$^-$ 373.0437, found 373.0436.

Analysis of **7** revealed > 94% purity:

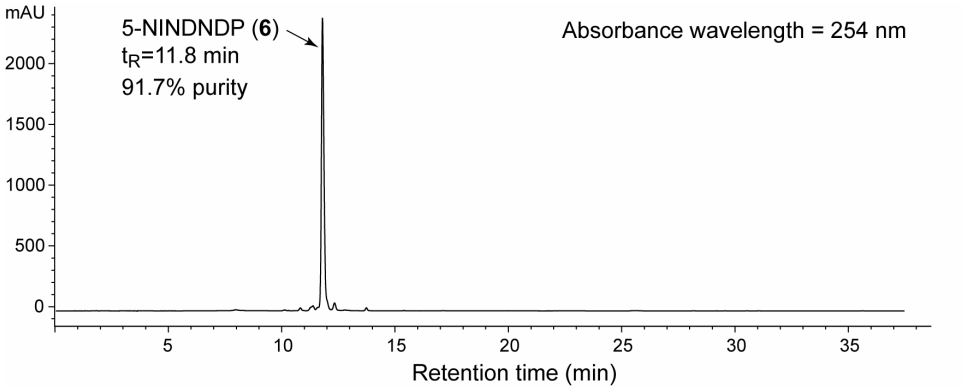



**1-β-D-ribofuranosyl-5-nitroindole diphosphate triethylammonium salt (6)**. To a solution of **7** (11 mg, 0.03 mmol) in DMF (1 mL) was added anhydrous $Bu_3N$ (8 µL, 0.03 mmol). The solution was stirred for 10 min then concentrated in vacuo. The resulting oil was redissolved in DMF (1 mL) and carbonyl diimidazole (27 mg, 0.17 mmol) was added.[17] The reaction mixture was stirred for 3 h at 23 °C, then quenched by addition of MeOH (10 µL). After stirring for an additional 30 min, a solution of anhydrous $H_3PO_4$ and $Bu_3N$ in DMF (0.5 M, 1.5 mL) was added. The solution was stirred for 12 h at 23 °C, then concentrated in vacuo. The crude reaction products were purified by preparative HPLC (HPLC purification method II)

yielding diphosphate **7** ($t_R$ = 9-10 min). The purified material was then concentrated in vacuo, redissolved in deionized $H_2O$ and lyophilized to dryness to afford yield diphosphate **6** (12 mg, 0.14 mmol, 50 % yield). $^1$H NMR (360.1 MHz, $D_2O$): *d* 8.45 (d, *J* = 2.2 Hz, 1H), 7.99 (dd, *J* = 2.2Hz, *J* =9.2 Hz, 1H), 7.66 (d, *J* = 3.5 Hz, 1H), 7.53 (d, *J* = 9.2 Hz, 1H), 6.72 (d, *J* = 3.4 Hz, 1H), 6.00 (d, *J* = 7.0 Hz, 1H), 4.40 (m, 1H), 4.17 (m, 1H), 3.95 (m, 2H), 3.00 (q, *J* = 7.3 Hz, 26H, TEAA salt), 1.80 (s, 2H, TEAA salt), 1.12 (t, *J* = 7.3 Hz, 37H, TEAA salt). $^{13}$C NMR (90.6 MHz, $D_2O$): *d* 141.6, 140.0, 128.5, 128.2, 118.3, 117.8, 110.1, 106.0, 88.0, 73.6, 70.4, 45.5 (TEAA salt), 8.2. (TEAA salt). $^{31}$P NMR (145.8 MHz, $D_2O$): *d* -7.38 (m), -10.19 (m). HRMS (ESI-) calcd for $C_{13}H_{15}N_2O_{12}P_2^-$ [*M*-TEAA+2H]$^-$ 453.0100. Found: 453.0078.

Analysis of **6** revealed > 91% purity:




**Biological Assay**

**Nucleotide incorporation by PV 3D$^{pol}$ in vitro.** PV 3D$^{pol}$ was expressed and purified as previously described.[18] Extension assays utilizing symmetrical primer-template substrates (s/s) were performed as described.[19] s/s RNAs were synthesized by Dharmacon, Inc. In brief, PV 3D$^{pol}$ was incubated with the appropriate s/s duplex for 90 s at 30 °C to allow formation of preinitiation enzyme-RNA complexes. Extension reactions were initiated by the addition of nucleotide and reactions were incubated at 30 °C. The initiated reaction contained 3D$^{pol}$ (5 μM), s/s RNA (1 μM), HEPES (50 mM, pH 7.5), 2-mercaptoethanol (10 mM), $MgCl_2$ (5 mM) and NTP. Reactions were quenched by addition of EDTA (final conc. = 50 mM, pH 8.0). For the experiment shown in Figure. 3, $MgCl_2$ was replaced with $MnCl_2$.[20,21] For all experiments, nonradiolabeled s/s "trap" (100 μM) was added along with initiating nucleotide to pre-

vent reinitiation of dissociated enzyme. Product was added to an equal volume of loading buffer (90% formamide, 0.025% bromphenol blue, and 0.025% xylene cyanol) and heated to 65 °C prior to loading on a denaturing polyacrylamide gel containing 23% acrylamide, TBE buffer (1X, 89 mM Tris base, 89 mM boric acid, and 2 mM EDTA), and urea (6 M). Electrophoresis was performed in TBE buffer (1X) at 80 W for ~2 h. Products were visualized by using a PhosphorImager (Molecular Dynamics). Quantitation was performed using ImageQuant software (Molecular Dynamics) and fit by nonlinear regression using KaleidaGraph 3.5 software (Synergy Software, Reading, PA).

**Stopped-flow kinetic assays used to determine $K_i$.** These assays follow the decrease in fluorescence generated as a result of nucleotide incorporation onto a s/s primer-template that contains the fluorescent probe 2-aminopurine (2AP) on the next position downstream of the templating base. The following RNA sequence, termed s/s-U-2AP, was employed: 5'-GC(2AP)UGGCCC-3'. All fluorescence experiments were performed using an SF-2001 model stopped-flow apparatus (KinTek Corp, Austin, TX) equipped with a recirculating water bath. The excitation wavelength was 313 nm, and the emission was captured above 370 nm using a cut-on filter (Chroma Technologies, Rockingham, VT). All reactions were run in HEPES buffer (50 mM, pH 7.5) containing 2-mercaptoethanol (10 mM), $MgCl_2$ (5 mM) and $ZnCl_2$ (60 μM). Reactions were performed at 30 °C. $3D^{pol}$ was diluted immediately prior to use in HEPES (50 mM, pH 7.5), 2-mercaptoethanol (10 mM), $ZnCl_2$ (60 μM), and glycerol (20 %). $3D^{pol}$-s/s complexes were assembled by mixing annealed s/s-U-2AP (1.0 μM) with $3D^{pol}$ (1.0 μM) in the reaction buffer for 3 min at 23 °C. This mixture was placed in one of the stopped-flow syringes and allowed to equilibrate for 2 min at 30 °C. The other stopped-flow syringe contained ATP alone (200 μM), or with ribavirin triphosphate (200 μM), **5** (200 μM), or **6** (200 μM) in the reaction buffer. The reaction was initiated by mixing the $3D^{pol}$-s/s complex with the nucleotide solution and recording the fluorescence decay for at least 5 times the reaction half-life (see Figure S2). After mixing, the reactant concentration was reduced by 50%. The experimental data shown in Figure S2 was normalized to the initial concentration of $3D^{pol}$-s/s complex present in the mixture. In contrast to nucleotide **5**, diphosphate **6**, or RTP, neither ribavirin (**1**), nucleoside **3**, nor UTP were inhibitors of $3D^{pol}$ under these conditions (data not shown). Figure 4 was obtained by fitting data shown in Figure S2 against a nonlinear

regression model to a single exponential using KaleidaGraph 3.5 software (Synergy Software, Reading, PA).

**Kinetic Simulation.** Kinetic simulations were performed by using KinTekSim Version 2.03 (KinTek Corp., Austin, TX). The experimental data shown in Figure S2 was fitted to the competitive inhibition mechanism shown in Figure S1. The values of $K_{d,app}$ and $k_{pol}$ for the uninhibited reaction to be used in the simulation were determined experimentally and are 66 μM for $K_{d,app}$ and 70 $s^{-1}$ for $k_{pol}$. In the simulation, the association rate constant for inhibitor binding was set at 10 $\mu M^{-1}\ s^{-1}$ and only the dissociation rate constant was varied. The agreement between the experimental data and kinetic simulations was determined by visual inspection.

**Cells and viruses.** HeLa S3 cells were maintained in DMEM/F-12 supplemented with 2% dialyzed fetal bovine serum and penicillin/streptomycin (Invitrogen). Nucleoside **3** was freshly suspended in 100% DMSO to 200 mM immediately prior to use. Ribavirin (a gift of Zhi Hong, Valeant Pharmaceuticals) was suspended in deionized water. For cytotoxicity studies, HeLa S3 cells ($1 \times 10^5$) were plated the day before in 24-well plates. The number of cells counted in controls after a 24 h incubation were assigned a value of 100. Cells were incubated with ribonucleoside **3,** ribavirin (**1**), sulconazole (**8**) or combinations thereof at 0.1 to 2 mM for 7 h at 37 °C. All wells were adjusted to a final concentration of 1% DMSO. Media was removed and cells were washed with 0.5 mL phosphate-buffered saline (PBS). Cells were allowed to grow for an additional 24 h in the absence of compounds. Cell monolayers were washed in PBS (0.5 mL), dissociated by treatment with trypsin (Invitrogen), and viable cells were counted by trypan blue exclusion using a hemacytometer. Error bars represent the standard deviation.

Infection with PV employed HeLa S3 host cells ($1 \times 10^5$) plated 1 day prior to treatment in 24-well plates. Cells were pretreated by addition of nucleoside at the specified concentration in fresh media adjusted to a final concentration of 1% DMSO. After a 1 h incubation at 37 °C, media was removed and cells were infected with PV ($1 \times 10^6$ PFU) in PBS (total volume = 0.1 mL). Plates were incubated for 15 min at 23 °C, PBS was removed by aspiration, and fresh, prewarmed (37 °C) media containing the specified amount of nucleoside was added. The infection was allowed to proceed at 37 °C for 6 h. Cells were washed with PBS and collected after treatment with trypsin. Cells were pelleted by centrifugation, resuspended in PBS (0.5 mL),

and subjected to 3 freeze-thaw cycles. Cell debris was removed by centrifugation and the supernatant containing the cell-associated virus was saved. Titer was determined by applying serial dilutions of supernatant to HeLa S3 monolayers (plated in 6-well plates 1 day before at $5 \times 10^5$ cells/well) and overlaying with growth media containing low melting point agarose (1%). Plates were incubated for 2-3 days at 37 °C, at which time the agar was removed and plaques were visualized by staining with crystal violet (1%) in aqueous ethanol (20%).

**X-Ray Crystallography Information for 3**[I]

**Ribonucleoside 3.** A clear needle shaped crystal of **3** ($C_{13}H_{14}N_2O_6$) obtained by vapor diffusion (methanol/pyridine/pentane) with approximate dimensions 0.10 x 0.16 x 0.50 mm, was used for the X-ray crystallographic analysis. The X-ray intensity data were measured at 95(2) K, cooled by Rigaku-MSC X-Stream 2000, on a Bruker SMART APEX CCD area detector system equipped with a graphite monochromator and a $Mo_{K\alpha}$ fine-focus sealed tube ($l$ = 0.71073 Å) operated at 1600 W power (50 kV, 32 mA). The detector was placed at a distance of 5.8 cm from the crystal.

A total of 1850 frames were collected with a scan width of 0.3° in ? and an exposure time of 10 s/frame. The total data collection time was about 8 h. The frames were integrated with the Bruker SAINT software package using a narrow-frame integration algorithm. The integration of the data using a orthorhombic unit cell yielded a total of 7707 reflections to a maximum ? angle of 28.28º (0.90 Å resolution), of which 2920 were independent, completeness = 97.4%, $R_{int}$ = 0.0401, $R_{sig}$ = 0.0454 and 2601 were greater than $2s(I)$. The final cell constants: $a$ = 5.7856(14), $b$ = 10.077(2), $c$ = 21.067(5) Å, $a$ = 90, ß = 90, ? = 90°, volume = 1228.2(5) Å$^3$, are based upon the refinement of the XYZ-centroids of 2710 reflections above $20s(I)$ with 2.240° < ? < 28.231°. Analysis of the data showed negligible decay during data collection. Data were corrected for absorption effects using the multiscan technique (SADABS). The ratio of minimum to maximum apparent transmission was 0.732661.

The structure was solved and refined using the Bruker SHELXTL (Version 6.1) Software Package, using the space group $P2(1)2(1)2(1)$, with $Z$ = 4 for the formula unit, $C_{13}H_{14}N_2O_6$ . The final anisotropic full-matrix least-squares refinement on $F^2$ with

[I] The small molecule X-ray crystallography facility was established using funds from an NSF Chemistry Research Instrumentation and Facilities grant (CHE-0131112).

193 variables converged at R1 = 4.20 %, for the observed data and *wR*2 = 10.05 % for all data. The goodness-of-fit was 1.037. The largest peak on the final difference map was 0.376 $e^-/Å^3$ and the largest hole was -0.190 $e^-/Å^3$. Based on the final model, the calculated density of the crystal is 1.591 $g/cm^3$ and F(000) amounts to 616 electrons.

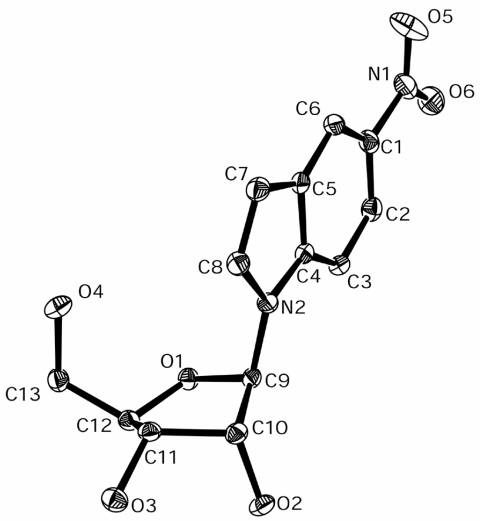



**Figure S3.** ORTEP structure of nucleoside **3** with explicit atom labels shown.

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